

PCR FOR DIAGNOSING TUBERCULOUS FISTULA IN ANO

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CERTIFICATE

This is to certify that the dissertation entitled **“WHAT IS THE PREVALANCE OF TUBERCULOUS FISTULA IN ANO IN A TERTIARY CARE CENTRE?; HOW USEFUL IS PCR AS A DIAGNOSTIC TOOL FOR TUBERCULOUS FISTULA IN ANO”** is a bonafide work of Dr. Partho Mukherjee in partial fulfillment of the requirements for the M.S. General Surgery (Branch I) examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in February 2010.

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INTRODUCTION

INTRODUCTION

Fistula in ano is a common surgical problem encountered in the out patient clinic. It is a condition which can be often diagnosed clinically with meticulous examination. It is mostly due to a benign cause which can be treated with surgical therapy. The treatment, although mainly surgical, involves consideration of certain systemic causes and prompt diagnosis and treatment of the same. Tuberculosis is one such ubiquitous cause, especially in developing countries. Various studies have found tuberculosis to be the cause to varying extents in cases of fistula in ano. However, the exact magnitude of the disease burden is not known.

Another problem with tuberculosis, in any form, is the difficulty in making a prompt diagnosis in order to start anti-tuberculous therapy. Even though a battery of tests exist, only some tests have stood the test of time and have been considered as gold standard. These tests are microscopy and culture demonstrating presence of *M. tuberculosis* in the specimen in question. However, even these tests have their shortcomings, a fact which has led to the quest for faster and more accurate tests. One such test is Polymerase Chain Reaction (PCR), a test which allows the amplification of a specific strand of DNA in a sample by enzymatic reaction, such that trace amounts of this DNA (which represents the Nucleic acid of the species to be detected) are picked up and amplified, thus confirming the diagnosis.

Fistulae caused by *M.tuberculosis* are difficult to diagnose because of low concentration of the acid fast bacilli in the tissue. Another factor is the overlap in the

histopathological appearance between tuberculosis and other infections causing granulomatous inflammation.

The present study tries to answer two of the questions regarding tuberculous fistula in ano. It tries to determine the prevalence of tuberculosis as a cause of fistula in ano. It also tries to look at how useful PCR is in diagnosing tuberculous fistula in ano.

STATEMENT OF STUDY QUESTION

WHAT IS THE PREVALANCE OF TUBERCULOUS FISTULA IN ANO IN A TERTIARY CARE CENTRE?

HOW USEFUL IS PCR AS A DIAGNOSTIC TOOL FOR TUBERCULOUS FISTULA IN ANO

AIMS & OBJECTIVES

AIMS & OBJECTIVES

Aims

1. To evaluate the prevalence of tuberculous fistula in ano in patients attending a tertiary care centre.
2. To determine the sensitivity and the specificity of PCR in the diagnosis of Tuberculous fistula in ano.

Objectives

To establish the disease burden of tuberculous fistula in ano among patients seen in the out patient department of a tertiary care centre (Christian Medical College) in Vellore.

To establish the role of PCR as a reliable method to diagnose tuberculous fistula in ano. The study aims at evaluating PCR as a tool for quick and reliable diagnosis of Tuberculous fistula in ano where the histopathology of the tract is indeterminate, thus saving the patient a 6-8 week wait before initiation of Anti - Tuberculous Therapy.

LITERATURE REVIEW

LITERATURE REVIEW

FISTULA IN ANO – AN OVERVIEW

One of the not so uncommon problems with which a patient presents to the general or colorectal surgeon in the out patient clinic is a protracted history of perianal discharge, often with associated history of having had multiple procedures for the same at another centre. Although the diagnosis of a fistula in ano for the above problem comes easily to a surgeon after a proper digital rectal examination, the treatment can be difficult. The entity of the fistula in ano has been afflicting man for long. Hippocrates, in about 430 B.C., suggested that the disease was caused by “contusions and tubercles occasioned by rowing or riding on horseback”. The fascination with anal fistula for more than 2,000 years is manifested by the numerous papers and books on the subject. (1)

SYMPTOMS

The presentation of fistula in ano is typical in most cases.

The most frequent presenting complaints of patients with an anal fistula are swelling, pain, and perianal discharge. The two former symptoms usually are associated with an abscess when the external or secondary opening has closed or has failed to develop. Most patients with an overt fistula have an antecedent history of abscess that drained spontaneously or for which surgical drainage had been performed. The discharge, however is mostly seropurulent, rarely blood tinged. The patients seeks medical help only if he has pain and swelling (? Abscess) or if the discharge becomes a

nuisance. The time period before the onset of the discharge and the presentation to the clinic varies with individuals' awareness about personal hygiene. This is the natural history of about 50% of the perianal abscess.(2)

PATHOLOGY

The pathology in fistula in ano is a fistulous tract between the anal canal (or the rectum) and the perianal skin which is lined with granulation tissue. The granulation tissue lining the tract may be representative of the pathology or may be non specific. The presence and the position of the openings of the tract (internal and external) can be one or more than one and the course of the tract between the two can be varied. This factor is used to classify the tracts and also helps in deciding on treatment.

CLASSIFICATION

A clear understanding of the anatomy of the anal canal and the related structures is essential to understand the pathogenesis and classification of fistula in ano We shall therefore briefly discuss the same before coming to the classification of anal fistulae.

ANATOMY OF THE ANAL CANAL AND THE MUSCLES OF CONTINENCE (3)

Anal canal

The muscular anal canal forms a sphincter at the distal end of the gastrointestinal (G.I.) tract. The adult anal canal is about 4 cm long. Posteriorly the anal canal is separated from the tip of the coccyx by fibro fatty and muscle tissue known as the ano coccygeal ligament. The ischiorectal fossae lie on either side of the anal canal and are

continuous with each other via the retrosphinteric space situated between the coccygeal attachment of the external anal sphincter below and the levator ani above. Anteriorly, the perineal body or the central tendon of the perineum separates the anal canal from the vagina or from the membranous urethra and bulb.

At the level of the dentate line, which is present in the zona columnaris, and represents the embryonic anal membrane site, marking the change in lining of the anal canal from columnar epithelium above it to the anal epithelium below it, are transverse folds of mucosa form a ring of anal valves. Immediately above these are shallow pockets (the anal sinuses or anal crypts). In the connective tissue zone between the sphincter ani muscles and the epithelial lining of the anal canal (zona columnaris) in this region, eccrine secreting glands, or “anal glands”, open into the crypts through ducts which have a glandular lining. (4) The anal glands, 5-10 in number, either branch in the submucosal plane, or, more frequently, penetrate the internal sphincter to end by branching in the intersphinteric plane between the internal and external anal sphincters. In some cases they extend only as far as the submucosa, while in others the gland enters the internal sphincter muscle and dilates in an ampulla like structure.

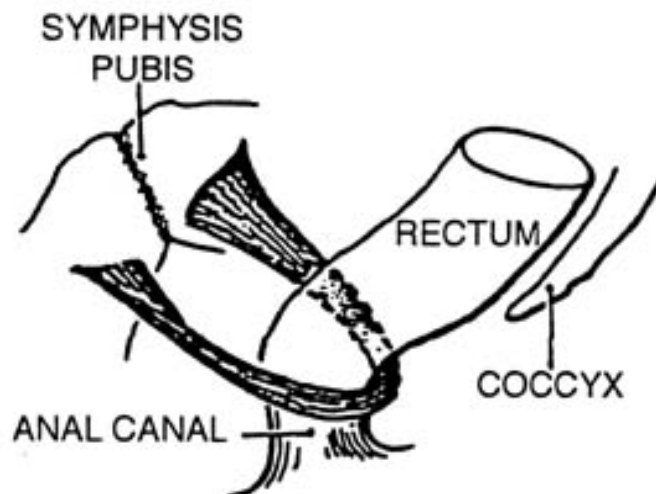
Significance of the dentate line

The dentate line marks the level of opening of the anal glands at the anal valves and thus is often the starting point of the abscess which lead to the formation of anal fistulae. The internal opening of the fistula, therefore often lies at the level of the dentate line.

The anal sphincters

The sphincter mechanism of the anal canal consists of the internal and external anal sphincters. The internal anal sphincter is a downward extension of the circular layer of the rectal muscle wall and is thus a smooth muscle tube under control of the autonomous nervous system. The external anal sphincter surrounds the internal and is continuous with the fibres of the levator ani muscle. It forms a skeletal muscle extension from the pelvic floor.

The upper part of the external sphincter, at the level of the anorectal junction, is the puborectalis muscle which forms a sling around the anorectal junction being attached anteriorly to the posterior aspect of the pubis. In the resting state, the anorectal tube is angled forward at this level and contraction of the puborectalis sling will increase this angle, an important factor in the continence mechanism. The anorectal ring is palpable on rectal examination.



While the internal sphincter is a well developed downward extension of the circular muscle layer of the rectum, the longitudinal layer contributes a far less discrete component. Partly muscle and partly fibrous tissue, it runs down to end as fibrous bands passing through the perianal fat and lower part of the external sphincter, to be attached to the skin. The fat of the perianal space is thus broken up into small loculi.

The levatores ani form the pelvic diaphragm supporting the pelvic viscera. The structures passing through the pelvic diaphragm lie within the sling of puborectalis. The levatores arise in continuity from the pelvic bone in front and from a thickening of the obturator fascia and the spine of the ischium laterally. They are inserted into the coccyx and the anococcygeal ligament posteriorly. The coccygeus muscle forms the posterior part of pelvic floor, its under surface being continuous with the sacrococcygeal ligament as seen from the perineal aspect. The nerve supply of the levator ani is from the 3rd and 4th sacral segments.

The intersphincteric space

The interval between the internal and external sphincters is known as the intersphincteric space. The anal intramuscular glands, which open into the anal crypts, pass through this space before penetrating into the internal sphincter.

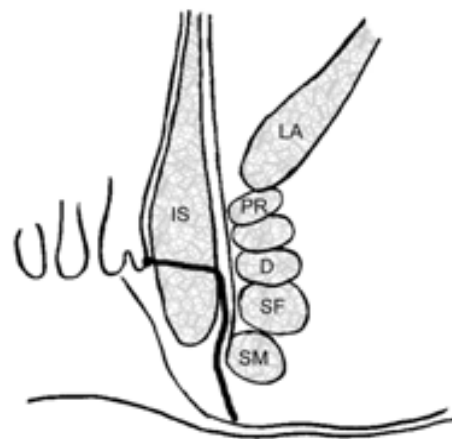
No essential nerves or blood vessels cross the space and the plane of dissection is fairly easily developed between the internal and external anal sphincters in an intersphincteric excision of the rectum.

Having thus discussed the relevant anatomy of the anal canal and the sphincteric muscles, it would be easier to understand the basis for classification of the fistula in ano.

The categorization of a fistula-in-ano is dependent on its location relative to the anal sphincter muscles according to Parks classification. (5,6):

- Submucous
- Intersphincteric
- Transsphincteric
- Suprasphincteric
- Extrasphincteric

The term submucous fistula is a misnomer because the condition does not fulfill the criterion for the definition of a fistula: a tract between two epithelial surfaces. These can be considered as intersphincteric fistulae.



Intersphincteric Fistula

The fistula forms between the internal and external sphincter.

The common type tracks down to present at the lower end of the internal sphincteric muscle as a perianal abscess.

Sometimes it tracks upwards and forms a second opening in the anus or rectum above that of the anal intramuscular glands. The track thus formed is not submucous (as was formerly thought) but intersphincteric.

The tract may extend into the pelvirectal space superiorly, and similarly a pelvic abscess may extend down the intersphincteric plane and present as a perianal abscess.



Transsphincteric Fistula

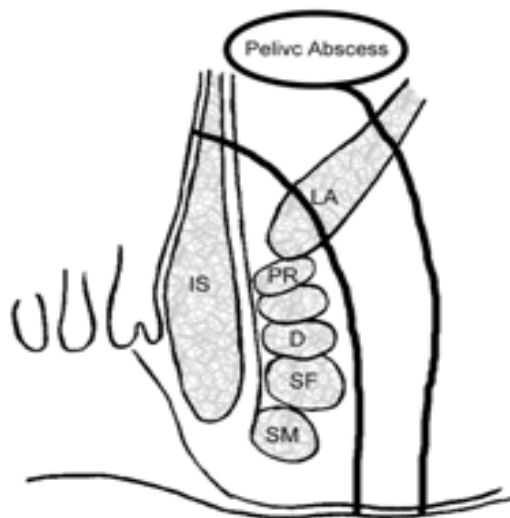
The infection extends from the intersphincteric plane at the mid anal canal level through the external sphincter into the ischiorectal fossa where it forms an abscess which then ruptures on to the perianal skin.

A secondary tract may extend upward through the levator ani and produce a pelvic abscess. The induration on rectal examination is felt at the anorectal ring.



Suprasphincteric Fistula

A pelvic extension of an intersphincteric abscess discharges through levator ani into the ischiorectal fossa. The tract curves above the puborectalis sling.



Extrasphincteric Fistula

IS - Internal sphincter
SM - Submucosal ext. sphincter
SF - Superficial ext. sphincter
D - Deep external sphincter
PR - Puborectalis muscle
LA - Levator ani muscle

The Extra sphincteric tract lies outside both the internal and the external sphincter muscles.

It may be caused by a trans sphincteric fistula which has entered the rectum at a higher level. It can be a sequelae to direct trauma to the perineum, Crohn's disease, or a carcinoma of the rectum.

A reasonable estimate of the distribution of the various types of fistulae in ano would be Intersphincteric 70%, Transsphincteric 23%, Suprasphincteric 5%, and Extrasphincteric 2%.

The term "complex" fistula is often used to describe "difficult to treat fistulae".(2) They are fistulae which pose a higher risk of sphincter injury and hence incontinence. They have been more objectively defined as a modification of the Parks classification. An anal fistula may be termed "complex" when:-

1. The track crosses >30 to 50 percent of the external sphincter (high-transsphincteric, suprasphincteric, and extrasphincteric),
2. is anterior in a female,
3. has multiple tracks,
4. is recurrent,
5. or the patient has preexisting incontinence, local irradiation, or Crohn's disease.

TREATMENT OF FISTULA IN ANO

The goals in fistula in ano treatment are the following:

1. To recognise and eliminate any septic foci in the perianal region and any associated epithelialised tracts.
2. To do so with minimal impairment of normal function.

Various techniques exist to treat fistulae. The merits and demerits of these have been discussed as follows.

Fistulectomy and Fistulotomy

A fistulotomy, includes defining the entire fistula track from internal opening to external opening with identification and obliteration of primary and secondary tracks. A fistulectomy on the other hand, involves coring out the tract in its entirety from the internal to the external opening. Fistulotomy is preferable to fistulectomy in spite of similar recurrence rates as the latter results in larger wounds with a longer healing time and higher rates of incontinence. The recurrence rate for fistulotomy is generally between 2 and 9 percent with a functional impairment generally between 0 and 17 percent.⁽⁷⁾ Patients with high openings, posterior openings, or fistula extensions are at risk to develop continence disorders after anal fistulotomy.⁽⁷⁾

Another procedure which is of use in decreasing wound healing time significantly is marsupialisation of the wound, a procedure which involves suturing of the wound edges with the fistula tract with interrupted absorbable sutures. This was found to

decrease the time of wound healing by up to 4 weeks with no significant increase in infection rates (8,9)

Seton

Fistula in ano can also be treated with the use of a seton and/or staged fistulotomy. A seton is a flexible foreign body (e.g., suture material, silastic vessel loop or a loop of linen) that is placed through the fistula track and secured to itself. Hippocrates was the first person to advocate the use of a seton (from the Latin seta, a bristle) in treatment by taking a very slender thread of raw lint, uniting it into five folds of the length of a span, and wrapping them round with a horse hair.²(1)

The use of setons is based on the assumption that they cause a chronic inflammatory reaction and consequent fibrosis that fixes and prevents retraction of the sphincter when it is divided. Based on whether the seton is tightened around the tissues, the setons can be classified as “cutting seton” and the “loose seton” which is used to perform a two-stage fistulotomy (TSSF).^(10,11,12) The cutting seton, by periodic tightening around the tissues encircled, gradually transects the muscle by pressure necrosis. In the TSSF, the seton is left loose around the tissue encircled by the fistula to stimulate fibrosis and facilitate drainage of sepsis. Division of the muscle encircled by the fistula is then performed as a second procedure. Proponents of the cutting seton argue that they do not need a second operation, whereas those in favour of the TSSF argue that the cutting seton produces unnecessary pain and divides the sphincter in an uncontrolled way.

Recurrence rates have been found to be nearly equal after use of cutting setons and TSSF (0-8%). Minor incontinence was also found to be present in about 50% of patients after TSSF and Cutting seton insertion. Major incontinence was present in about 5% of patients with the use of TSSF and of about 10% patients after use of cutting setons. It is therefore evident that the TSSF is just as effective in treatment of fistula as compared to the more painful cutting seton. (11,12,13) The rates of recurrence in cases of Crohn's disease have been found to be higher (upto 39%) (14).

Mucosal flap advancement

Another modality of treatment of fistula in ano includes what is called the mucosal flap advancement. The fistulous tract, running from the external opening to the external anal sphincter is excised as well. The tract running through the sphincters is curetted. The defect in the internal anal sphincter is then closed with absorbable sutures. The next step involves covering the internal opening with a mucosal or a skin based flap. There are two ways of raising flaps, the first being from the mucosa proximal to the internal opening which is advanced distally, taking care to include the muscularis propria with it. The incision begins distal to the internal opening, and the flap is mobilized, excising the scar. Some suggest an oblique incision, but this risks ischemic necrosis at the apex. Others prefer mobilization in a circumferential fashion, incorporating as much as 50% of the rectum. The dissection can be facilitated by means of infiltration with a dilute epinephrine solution. (15)

The concept of endoanal or endorectal mucosal advancement has evolved into three principal methods: vertical tongue flaps, semilunar lip flaps, and circumferential

tubal or sleeve flaps. The second method involves an (inverted) U shaped flap including perianal skin and fat, is created, taking care not to undermine the flap to prevent ischemia. The flap is designed in such a way that the base of the flap is approximately twice the width of its apex. The flap is advanced into the anal canal and sutured to the mucosa and underlying internal anal sphincter in a single layer, proximal to the closed internal opening, using interrupted, absorbable sutures. The perianal wound is left open.(15) .

The mucosal advancement flap has a cure rate of upto 80-90 percent in most studies(16,17,18) . Schouten showed in his studies that use of anal advancement flaps had a reduced continence to flatus in 38% and faeces 12%. (19)

Fibrin glue

Another way of treating fistula in ano is to inject into the tracts (after curretting the granulation tissue) fibrin glue which initiates healing , with minimal surgical trauma. This procedure gives an over all healing rate of 69% for all fistula in ano.(20,21) Another study found that the fibrin glue was 33% succesful and 14% succesful in closing primary and recurrent fistula respectively(22).

When compared with fistulotomy, the fibrin glue was found to be more effective in treating complex as compared to simple fistulae, healing 69% of the complex and only 50% of the simple fistulae as compared to 13% and 100% healing rates in the corresponding fistulotomy arms. (23)

Fibrin glue treatment is simple and repeatable; failure does not compromise further treatment options; and sphincter function is preserved.(23)

It has also been demonstrated that combining the fibrin glue with an advancement flap shows worst outcome as compared to the fibrin glue or the advancement flap alone.(24)

Fistula plug

The fistula plug is a bioprosthetic material which is inserted into the tract to promote occlusion of the tract and healing. Although initial results were promising, on long term follow up the fistula plug has shown poor healing rates of only 13-24% with high incidence of post operative local sepsis. (25,26,28)

To summarize, for the treatment of fistula in ano, one should keep in the mind that, Marsupialization after fistulotomy reduces bleeding and allows for faster healing. Results from small trials suggest flap repair may be no worse than fistulotomy in terms of healing rates but this requires confirmation. Flap repair combined with fibrin glue treatment of fistulae may increase failure rates. (20)

In keeping with the goals mentioned above the principles of operative management are as follows:-

Identify the tract.(using a probe)

Incise the tract (using electrocautery) or debride and obliterate with fibrin glue, or place a seton, or close the internal opening with a mucosal advancement flap.

Excise a portion for biopsy material (if considered potentially useful).

Pack with Vaseline Gauze after hemostasis is secured

Following this the patient is advised to have regular sitz baths with use of laxatives and local anaesthetics.

It must be understood that no single technique can be used to treat every fistula. The key point is the surgeon's discretion in deciding how much of the sphincter if divided will be a reasonable trade off between post operative healing rates and functional detriment. During division of the sphincter, if needed, the surgeon needs to keep in mind the presence of Crohn's disease or previous radiation therapy. Other factors which are important in deciding on post operative functional status are

1. preexisting incontinence
2. previous mechanical sphincter injury,
3. an anterior location in females,
4. stool consistency, and finally
5. the patient's tolerance of potential imperfections in their continence.

Complications of fistula surgery are many and include fecal soilage, mucous discharge, varying degrees of incontinence (gas and/or stool), and recurrent abscess and fistula.

ETIOLOGY OF FISTULA IN ANO

The etiology of fistula in ano can be classified into the following heads

Cryptoglandular fistula in ano	- 90%
Specific causes (10%)	- Crohns Disease, Tuberculosis, Malignancy
Others (<1%)	- Sarcoidosis, Syphilis, Venereal lymphogranuloma and Actinomycosis

It would be safe to say that about 90% of the fistula in ano can be classified as cryptoglandular in origin. About 4-10 mucin producing anal glands are present at the level of the dentate line, draining into the anal crypts.(2) Fecal plugging of the ducts leads to obstruction and subsequent abscess formation. The abscess may spread through and in between the sphincters and track along the muscular, fascial and fatty planes around the anus at any anatomical level vertically, horizontally or circumferentially. This process represents the cryptoglandular theory of anal sepsis. Robinson and Seow have suggested that the description of the anal glands by Chiari in 1878 and the subsequent histological studies of Parks in 1961 contributed to the acceptance of the cryptoglandular theory as the most common cause for anal sepsis.(28,30)

Even though the majority of the cases of fistula in ano are caused by a benign cause, there is a small but definite proportion of anal fistulae which owe their origin to a more sinister cause and require accurate diagnosis, differentiation and management to

achieve healing. In a study with 233 patients who underwent operations for ano rectal sepsis, 11.6% were found to harbor systemic disease.(31)

These not so uncommon causes of fistula in ano which need to be considered and treated on a systemic basis include Crohn's disease and tuberculosis and malignancy. The other rare causes of fistula in ano are such as sarcoidosis, syphilis, venereal lymphogranuloma and actinomycosis should also be considered.

One of the not uncommon causes in India is Tuberculosis. Anorectal tuberculosis, although considered to be rare, is a cause of considerable importance because of the fact that the fistulae caused by M. tuberculosis are 1) Difficult to diagnose and 2) will not heal unless the diagnosis is made promptly and treatment with anti tuberculous drugs prescribed for an adequate duration.

Before we consider the various aspects of tuberculosis as a cause of fistula in ano, a brief description of gastrointestinal tuberculosis will help us in understanding the burden and the etiopathophysiology of the disease.

GASTROINTESTINAL TUBERCULOSIS

Tuberculosis (TB) can involve any part of the gastrointestinal tract from mouth to anus, the peritoneum and the pancreatobiliary system. It frequently mimics other common and rare diseases posing difficulties in its diagnosis and prompt treatment. TB of the gastrointestinal tract is the 6th most frequent form of extra-pulmonary in India. (32)

The other forms of extrapulmonary tuberculosis (EPTB) tuberculosis, which are more common than gastrointestinal TB, in decreasing order of their prevalence are

lymphatic , genitourinary , bone and joint , miliary and meningeal tuberculosis. The manifestation of extrapulmonary manifestations of tuberculosis have increased from 16% to 21% between 1993 and 2005 in the USA, according to the Centers for Disease Control and Prevention.(33)

Gastrointestinal tuberculosis accounts for about 10-15 percent of the cases of tuberculosis. Although there have been reports of intestinal involvement in upto 55-90 percent of cases with pulmonary tuberculosis in the pre anti tuberculosis therapy era, a study (based on autopsies) done between 1964 to 1970 showed presence of abdominal tuberculosis in 3.72% of cases with pulmonary tuberculosis.(32)

Among the various forms of gastrointestinal tuberculosis, those affecting the anus often manifest as anal fistulae.

TUBERCULOSIS IN FISTULA IN ANO

Tuberculous involvement of the anus is rare(29). According to some authors, anorectal tuberculosis is mostly associated with active pulmonary tuberculosis. The postulated mechanisms by which tubercle bacilli reach the perianal region are: 1) Hematogenous spread from the primary lung focus in childhood with later reactivation; 2) ingestion of bacilli in sputum from active pulmonary focus; 3) direct spread from the adjacent organs; and 4) through lymph channels from infected nodes.

Tuberculous origin must be considered when the cause of perianal ulcers is unclear.

The percentage of cases of fistula in ano caused by tuberculosis varies, with figures varying from 0.7 % to 21 % of the cases of fistula in ano. (34,35,36,37,38,39)

Anorectal tuberculosis has been classified by Nepomuceno et al into 4 categories: ulcerative, verrucous, lupoid, and miliary. The most common type is ulcerative tuberculosis, which usually is a result of a primary source often found in the lung or intestines. The verrucous type is characterized by a warty appearance. The lupoid type starts as a reddish-brown nodule and eventually forms an ulcer in its center, whereas miliary lesions are associated with a systemic process. Fistulae may develop from any of these initial types.(40). Other atypical presentations are recurrent perianal growth, anal fissure, anal strictures, and a rectal submucosal tumor.(35)

Most frequently encountered anorectal tuberculous lesions are suppurations and fistulae. Other presenting features may be anal pain, fever and cough, anal or perianal ulcer with purulent exudates and a nonhealing wound around the anus. (35,37)

Nearly all tuberculous fistulae are complex, and secondary tracks or additional complicating features are commonly found even at first presentation. (41)

Tuberculosis can be present in HIV-positive patients.(42) The incidence and severity of ano-perianal tuberculosis are increasing with increasing incidences of HIV infection. Few other clinical conditions mimicking tuberculosis are hidradinitis suppurativa, bartholinitis, radiation injuries, lymphomas and antibiomas.

INVESTIGATIONS FOR TUBERCULOSIS IN FISTULA IN ANO

The key factor in the diagnosis and therefore treatment of tuberculosis, more so in its extra pulmonary and smear negative forms, is that of time. Although early diagnosis of infection is important before the use of antituberculous chemotherapy clinical diagnosis is usually dependent on microscopic detection using Ziehl-Neelsen stain and mycobacterial culture, but the sensitivity and specificity of these two methods are low. Various tests have been introduced so as to try and diagnose a case of tuberculosis as early as possible, with a reliable degree of specificity and sensitivity so as to start ATT promptly.

Some of the tests have been discussed as follows:-

The diagnostic tests available for the detection of tuberculosis in the present day are microscopy, culture, drug susceptibility tests, rapid tests like NATS or Phage Assays, or immune based tests like tuberculin, Interferon Gamma release assays.

MICROSCOPY

Slides are stained with carbolfuchsin (e.g. Ziehl-Neelsen (ZN) stain) or fluorochrome dyes (auramine stain) and examined with light and fluorescent microscopy. The advantages of microscopy for detection of M. tuberculosis are many. The smears are cheap, rapid and detects the most infectious cases. In areas of high TB prevalence microscopy is considered relatively specific for Mycobacterium tuberculosis (90 - 95%). In Ziehl-Neelsen staining, the smear is covered with carbol fuchsin dye for a few minutes. After heating, rinsing with water and the acid-alcohol

treatment, the smear is counterstained with another dye, methylene blue (to colour the background of the smear for contrast) and then rinsed again (WHO 2004). Once dried, if there was a substantial concentration of TB bacilli in the specimen, some of the bacilli on the slide should show up under the microscope as red, characteristically rod-shaped organisms, against the blue. The lab technician must meticulously examine each slide and then record the number of organisms observed (grading the burden — which can predict severity of disease in HIV-negative people). From sample collection until microscopic examination, the process takes at least a couple of hours. The microscopic examination itself is a labour intensive and time consuming procedure. However, even though the technique is simple enough to be performed even in settings with rudimentary facilities, it is not very sensitive. There has to be a very high concentration of bacilli in the specimen in order for the lab technician to detect the ten or more organisms needed for a clear, positive result. Even a skilled eye working in a well-maintained laboratory is unlikely to detect TB when there are fewer than 5000–10,000 bacilli per ml of sputum. Such a high of a burden of bacilli is typically only found in adults with advanced pulmonary disease — and rarely in earlier disease, or when the disease is active in other parts of the body (extrapulmonary TB), in people with HIV or in children. Therefore, for paucibacillary tuberculosis, AFB smear microscopy is limited by its poor sensitivity (45%–80% with culture-confirmed pulmonary TB cases) and its poor positive predictive value (50%–80%) for TB in settings in which nontuberculous mycobacteria are commonly isolated (43,44).

MYCOBACTERIAL CULTURE

Apart from the demonstration of AFB positive bacilli by direct microscopic examination of the specimen, a positive culture proving the presence of AFB (M. tuberculosis) remains the gold standard for the diagnosis of TB, particularly in immunocompromised smear-negative patients. The sensitivity of AFB smear and culture, though, are low; cultures grow mycobacteria in 39 to 80% of cases. (39) The advantage that AFB culture has over microscopy is that it is much more sensitive than the microscopy, being able to detect as few as 10 bacteria/ml of material (46,47). There is also the need for precise speciation which culture addresses, also providing live organisms for drug susceptibility testing and genotyping of the organisms. Therefore, it is advisable to inoculate (after appropriate digestion and decontamination, if required) all clinical samples. In general, the sensitivity of culture is 80-85% with a specificity of approximately 98% (43,44).

The diagnosis of tuberculosis disease is mainly bacteriologic in adults.

Three different types of traditional culture media are available:

1. 1.Egg based (Lowenstein-Jensen),
2. 2.Agar based (Middlebrook 7H10 or 7H11 medium), and
3. Liquid (Middlebrook 7H12 and other commercially available broths).

Each can be made into selective media by adding antibiotics. Of the solid media, growth of mycobacteria tends to be slightly better on the egg-based medium but more

rapid on the agar medium. Growth in liquid media is faster than growth on solid media. However, liquid media can be used for primary isolation of mycobacteria from nonsterile sites only if supplemented with an antibiotic cocktail.

A major improvement in mycobacteriology has been the development of commercial broth systems for mycobacterial growth detection. Automated culture systems in this line are the Liquid-based commercial culture systems BACTEC460 (Becton Dickinson Microbiology Systems, Sparks, MD), mycobacterial growth indicator tube (MGIT) systems, ESP (Extra Sensing Power) Myco-ESPculture System II (Trek Diagnostic Systems, Inc., Westlake, OH), and BacT/ALERT MB Susceptibility Kit (Organon Teknika, Durham, NC). These use Middlebrook 7H12 media with added material for detection of mycobacteria (radiometric or calorimetric systems). The chief advantages of these are the more rapid turnaround time (10 - 14 days for growth, compared with ≥ 3 weeks on solid media) and improved sensitivity. However, the contamination rates are higher with some of the liquid-based systems. At least one container of solid medium should be inoculated and used in conjunction with broth culture systems. Egg-based media such as Lowenstein-Jensen slants are an important backup for rare *M. tuberculosis* strains that may not grow on the other media. Automated liquid systems should be checked at least every 2-3 d for growth while solid media should be checked once or twice a week. Mycobacterial growth observed on solid culture media should be quantified. Growth in liquid culture systems cannot be similarly quantitated although a qualitative measure of organisms in the inoculum can be made by noting the time required for liquid culture to turn positive.

Phage-based assays

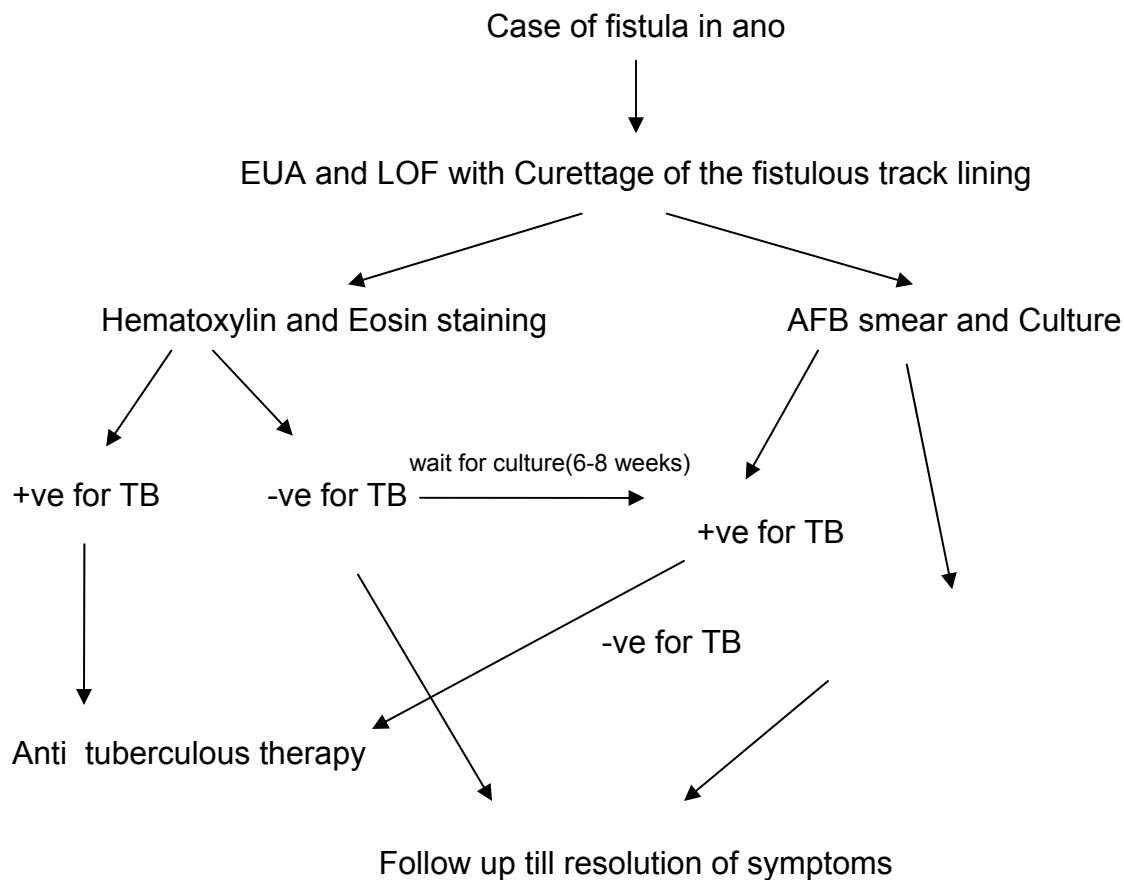
These assays use mycobacteriophages to infect any viable *M. tuberculosis* present in a sample.

The remaining extracellular phages are killed by the addition of a viricidal agent, and the protected intracellular phages are subsequently detected as clear areas or plaques in a lawn of rapidly growing indicator mycobacteria. An alternative, more rapid detection system uses genetically engineered bacteriophages with a luciferase reporter gene that allows detection through the emission of light. The assay can be modified for the rapid testing of resistance to Rifampicin. Although these tests show good sensitivity and specificity when used on culture isolates, the performance of these tests is diminished when used on field samples directly. This limits the use of these tests.

Tuberculin skin test

The tuberculin skin test (TST), involving the intra-dermal inoculation of a known amount of tuberculin protein, followed by the recording and interpretation of the subsequent induration, measures some aspects of the host's cell-mediated immune response to TB. The interpretation of the test is complicated by cross-reactions in persons either vaccinated with BCG or exposed to environmental mycobacteria, and false-negative results can occur owing to anergy. Most importantly, the TST does not differentiate latent infection from TB disease although it is still often used as an adjunctive diagnostic test in the paediatric setting.

The present day algorithm which is followed for a patient who presents to the out patient clinic in our institution to rule out, diagnose and if required, treat tuberculous fistula in ano is as follows: (EUA and LOF – Examination under Anaesthesia and Lay open of Fistula)



However, there is an inherent problem with the above algorithm, one of diagnosing tuberculosis with certainty in cases where 1) The AFB smear from the tissue is negative and 2) when the histopathological examination is not conclusively diagnostic of tuberculosis. In these cases, the clinician needs to wait for a period of about 6-8 weeks for the M.tuberculosis culture to rule out or rule in tuberculosis before starting treatment.

The main difficulty with extrapulmonary specimens is that they yield very few bacilli and consequently are associated with low sensitivity of acid-fast bacillus (AFB) smear and culture. Acid-fast staining was positive in fewer than 10% of patients in most reports, whereas pleural fluid cultures for *M. tuberculosis* were positive in up to 12 to 70% of cases and pleural biopsies revealed granulomas in 50 to 97% of patients with tuberculous pleural effusion. (45)

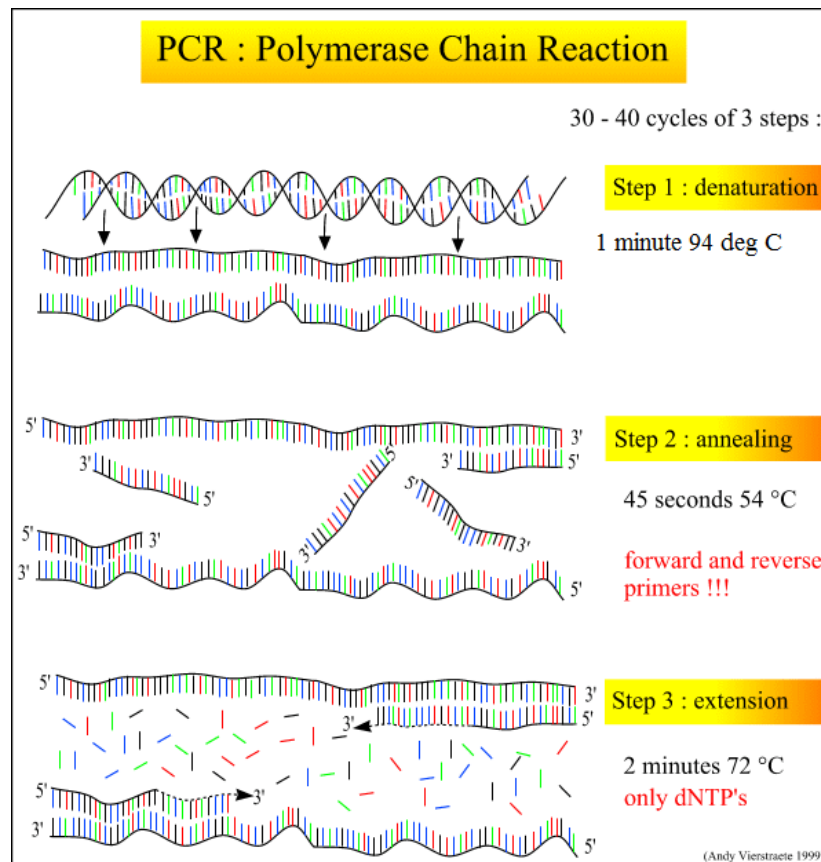
Of all the forms of gastrointestinal tuberculosis, tuberculous fistula in ano is probably one of the most paucibacillary types. Also, various other diseases, particularly Crohn's disease show granulomas on histology, with the culture or smear not showing any evidence of tuberculosis, confounding the picture. In view of the fact that Tuberculous fistula in ano is paucibacillary and the attendant difficulties in detection, a test that increases the number or yield of bacteria is required. The Nuclear Amplification Tests (NATS), in this regard have emerged as test with 1) greater positive predictive value (>95%) with AFB smear-positive specimens in settings in which nontuberculous mycobacteria are common and 2) ability to confirm rapidly (24-48 hours) the presence of *M. tuberculosis* in 50%-80% of AFB smear-negative, culture-positive specimens (48,49,50,51,52). Compared with culture, NATs can detect the presence of *M. tuberculosis* bacteria in a specimen weeks earlier than culture for 80%-90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture (48,49,50). The Polymerase Chain Reaction (PCR) is one such NAT.

The basic PCR principle is simple. As the name implies, it is a chain reaction: One DNA molecule is used to produce two copies, then four, then eight and so forth. This continuous doubling is accomplished by specific proteins known as polymerases,

enzymes that are able to string together individual DNA building blocks to form long molecular strands. To do their job polymerases require a supply of DNA building blocks, i.e. the nucleotides consisting of the four bases Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). They also need a small fragment of DNA, known as the primer, to which they attach the building blocks as well as a longer DNA molecule to serve as a template for constructing the new strand. If these three ingredients are supplied, the enzymes will construct exact copies of the templates. This process is important, for example, when DNA polymerases double the genetic material during cell division. Besides For the copying procedure only a small fragment of the DNA section of interest needs to be identified. This then serves as a template for producing the primers that initiate the reaction. It is then possible to clone DNA whose sequence is unknown. This is one of the method's major advantages. The enzyme which is used to accomplish the DNA polymerization at such high temperatures is from the bacillus *Thermus aquaticus*, and its heat-stable polymerase, called Taq polymerase.

It uses repeated cycles, each of which consists of three steps: 1. The reaction solution containing DNA molecules (to be copied), polymerases (which copy the DNA), primers (which serve as starting DNA) and nucleotides (which are attached to the primers) is heated to 95°C. This causes the two complementary strands to separate, a process known as denaturing or melting. 2. Lowering the temperature to 55°C causes the primers to bind to the DNA, a process known as hybridization or annealing. The resulting bonds are stable only if the primer and DNA segment are complementary, i.e. if the base pairs of the primer and DNA segment match. The polymerases then begin to attach additional complementary nucleotides at these sites, thus strengthening the

bonding between the primers and the DNA. 3. Extension: The temperature is again increased, this time to 72°C. This is the ideal working temperature for the polymerases used, which add further nucleotides to the developing DNA strand. At the same time, any loose bonds that have formed between the primers and DNA segments that are not fully complementary are broken. Each time these three steps are repeated the number of copied DNA molecules doubles. After 20 cycles about a million molecules are cloned from a single segment of double stranded DNA. The temperatures and duration of the individual steps described above refer to the most commonly used protocol.(52,53,54)



Certain PCRs are designed to amplify nucleic acid regions specific to the *Mycobacterium tuberculosis* complex. These tests can be used directly on clinical

specimens (eg. Sputum). PCRs are also available as commercial kits which include reagents to extract and then amplify nucleic acid chains when subject to a certain set of conditions. The in-house base Polymerase chain reaction (PCR) assays vary widely in their protocols and methods.

The literature on NAT demonstrates high specificity for pulmonary and extrapulmonary TB. However, data on extra pulmonary forms of TB are limited. The high specificity of NATs helps to rule in TB in cases with high pretest probability. (55)

In contrast to the specificity, the sensitivity (The ability of the test to rule out the disease) is lower and highly variable. The sensitivity is especially low in paucibacillary forms of tuberculosis (namely extra pulmonary forms of TB). The sensitivity of NATs has been the highest in smear positive specimen. Therefore, the negative test does not rule out the disease. There have been reports of use of the IS6110 as an amplification target and the use of nested PCR with higher accuracy. (56,57)

Various samples have been used to try and diagnose with a greater accuracy , or to evaluate the role of PCR as a tool for the diagnosis of Extra Pulmonary Tuberculosis.

Certain reports state the sensitivity and negative predictive value of peripheral blood samples from 38 EPTB and 89 non tuberculous subjects as 60.53% and 76.92% respectively, which is superior to the present gold standard of mycobacterial culture (10.53 and 72.36%). However, 43.82% of non tuberculous subjects gave positive results with the PCR, thus mitigating the clinical utility of this test.(58)

Other studies have used stool samples as specimens to diagnose intestinal tuberculosis and have found a sensitivity, specificity, positive predictive value and a negative predictive value of 88.8%, 100%, 100%, and 93.7% respectively. (59)

Urine has been used as a specimen in some studies, with similar results.(60,61)

The use of tissue from the site of suspected lesion as a specimen for the PCR has been tried. The IS6110 is a 123 base pair (bp) restriction fragment length polymorphism (RFLP) fragment which is specific for *Mycobacterium tuberculosis* as it is repeated multiple number of times in the species DNA. There have been various studies which have used this base pair sequence as a specific marker for the detection of the *Mycobacterium tuberculosis* genome in the specimen with varying degrees of success. Studies involving direct detection of *Mycobacterium bovis* in bovine lymph node samples (with macroscopic features of tuberculosis) using PCR have shown concordance in the positive rates with the PCR and the Cultures. (62)

Another study used 191 non-repeated clinical samples of EPTB and 17 samples from non-tuberculous cases as controls. All the samples were stained for acid fast bacilli (AFB) and 143 samples were processed by culture for *M. tuberculosis*. All the samples were processed for PCR amplification with primers targeting 123 bp fragment of insertion element IS6110 of *M. tuberculosis* complex. Of the 191 samples processed, 34 (18%) were positive by smear for AFB. Culture for AFB was positive in 31(22%) of 143 samples processed. Either smear or culture for AFB was found positive in 51(27%) samples. Of the 191 samples processed 120 (63%) were positive by PCR. In 140 samples, wherein both the conventional techniques were found negative, 74 (53%)

samples were positive by PCR alone. Among 51 samples positive by conventional techniques, 46 (90%) were found positive by PCR. (63,64)

Another study which used sputum PCR and compared it with conventional diagnostic methods (smear and culture) found a sensitivity, specificity, positive predictive value and a negative predictive value of 85%, 97%, 94%, and 94% respectively for the IS6110 123 base pair insertion sequence.

Therefore, the use of PCR with the IS6110 123 bp insertion sequence as the target sequence to diagnose the presence of M. tuberculosis in a given sample does show promise. However, its role in aiding the diagnosis of tuberculous fistula in ano remains largely unknown. This study aims at throwing light on the same.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study design – The study was a prospective blinded crossectional study with the evaluation of a diagnostic tool.

Patients, clinical information, and clinical specimens.

Patients with fistula in ano were drawn from those attending the Out patient department of General surgery Units II and V of the Christian Medical College, Vellore over a period of 20 months (from September 2007 to April 2009). A detailed clinical history, physical examination, baseline laboratory investigations to asses fitness for surgery were conducted for all patients.

At the time of the operation, a sample of the curetted fistulous tract was obtained and was cryo preserved at -196 degree C for later use. Bits from the same fistulous tract curettage were sent for AFB smear and culture examination and hematoxylin and eosin histopathological examination (HPE).

The HPE reports were followed up subsequently.

The AFB culture were followed up for a period of 10 weeks for growth of Mycobacterium tuberculosis.

The cryopreserved specimens were subjected to PCR for the presence of IS6110 amplification target.

The clinical diagnosis was not known to the laboratory personnel conducting the PCR tests.

Sample size

The sample size was calculated by using the prevalence as established by a previous retrospective study done in the Department of General Surgery Unit V which had determined the prevalence of tuberculous fistula in ano as 6.6%. (ref. unpublished data)

The formula used for calculation of the sample size was $4pq/d^2$, where P is the expected prevalence, Q is (1-P), and D is the degree of accuracy expected out of the study. It was decided to do the study with an expected degree of accuracy of +/- 3%, as this would need a sample size which could be completed in the study period. Therefore, using this formula, a sample size of 263 was calculated.

STATISTICAL ANALYSIS OF DATA

The patients whose samples are used in the study were analyzed by way of their distribution of age, sex, number of fistulae found on clinical examination and total number of procedures done for the same complaints.

The prevalence of tuberculosis was thus calculated based on the number of patients positive for the gold standard tests divided by the total number of patients examined over the study period.

The number of samples which were positive for AFB smear and/or are positive for tuberculosis on histopathological examination were grouped together as positive for the gold standard test.

The number of patients who were positive for Mycobacterium tuberculosis genome on PCR examination were considered to be test positive for the diagnostic tool being evaluated.

A two by two table as follows was thus constructed with the above data.

Total number of cases examined = X

Total number of cases positive for histopathology or AFB culture = Y

Total number of negative cases = Z

AFB C/S AND/OR HPE		+ VE	- VE
+ VE PCR		A (TP)	B (FP)
		C (FN)	D (TN)
- VE			

$$Y + Z = X$$

The PREVALENCE of tuberculous fistula in ano

No. of HPE and or AFB culture +ve cases (Y)

Sample size attained in the study period (X)

The SENSITIVITY of PCR

The positive cases in PCR (Test +ve) (A)

The cases +ve for HPE and or AFB culture (disease +ve) (A+C)

The SPECIFICITY of PCR is

The number of cases negative for PCR (Test -ve)(D)

Number of cases -ve for HPE and or AFB culture(Disease -ve)(B+D)

The POSITIVE PREDICTIVE VALUE is

The number of cases with the actual disease (A)

The number of cases with Positive PCR (A+B)

The NEGATIVE PREDICTIVE VALUE is

The number of cases with no disease (D)

The number of cases with negative PCR(C+D)

RESULTS

RESULTS

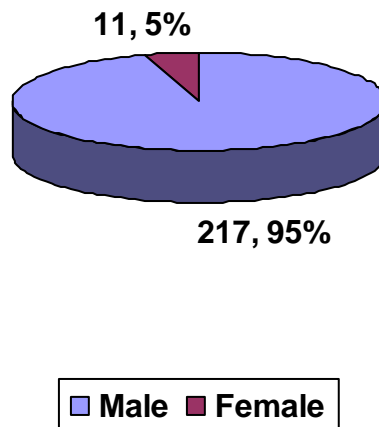
TOTAL CASES

A total of 231 samples were included in the study. These samples were derived from 228 patients.

AGE

The mean age of the patients was 42 years, with a range of 12-69 years of age.

SEX

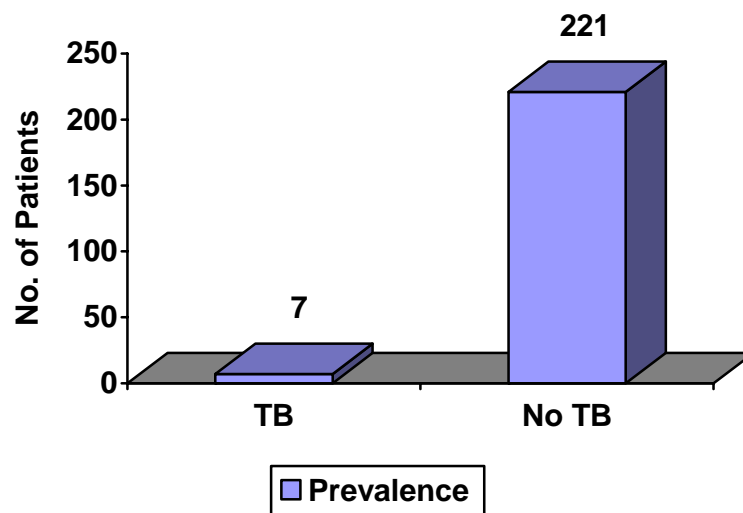


The majority of the patients were males and only 11 (4.8%) were females.

OTHER VIRAL INFECTIONS

None of the patients were HIV positive. However, 3 patients were HbsAg positive and one patient was HCV positive.

TUBERCULOSIS



7 patients were diagnosed to have tuberculous fistula in ano based on either histopathology or AFB culture. Therefore, a prevalence of 3.07% was determined.

DURATION OF SYMPTOMS

The mean duration of symptoms before the patients presented was 31 months with a range of half a month to 20 years.

NUMBER OF TRACTS

58 patients had greater than one tract at the time of presentation.

NUMBER OF PROCEDURES

50 patients had undergone three or more perianal procedures at the time of presentation at various centers including ours. However, only 12 of these patients had more than one tract.

PREVELANCE OF TUBERCULOSIS

Tuberculosis was diagnosed in 7 out of the 228 patients. This gave a prevelance of 3.07 % for tuberculosis among patients of fistula in ano. All these patients were males with an average age of 44 years of age.

NUMBER OF TRACTS IN TUBERCULOSIS PATIENTS

Most of the patients (n= 6) diagnosed to have tuberculosis had single tracts.

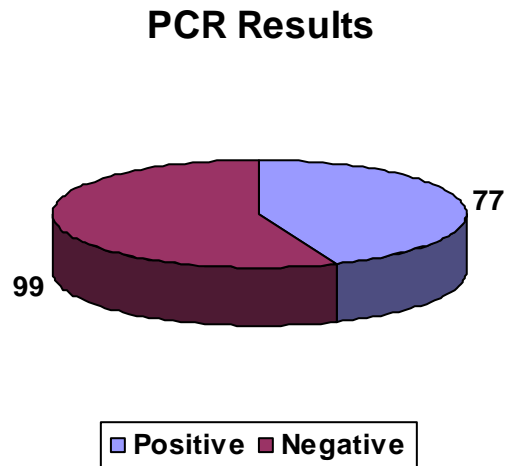
DURATION OF SYMPTOMS AMONG THE TUBERCULOUS FISTULA IN ANO PATIENTS

The average duration of symptoms among the tuberculous fistula in ano patients was 18 months, with a range of 4 months to 3 years.

BIOPSY AND CULTURE POSITIVE

3 patients were biopsy positive. 3 patients were culture positive. One patient was biopsy and culture positive.

PCR POSTIVES



A total of 176 samples were subjected to PCR, of which 77 samples were found to be PCR positive for tuberculosis. Six of these samples were positive for tuberculosis by culture or histopathology.

OTHER BIOPSY

The other biopsy reports encountered were adenocarcinoma (1 patient), Crohn's disease (1 patient) , and granular cell tumour (1 patient).

THE TWO BY TWO TABLE WAS THUS FORMED :-

		AFB CULTURE AND/OR HISTOPATHOLOGY POSITIVE	
		+	-
PCR	+	4 TP	73 FP
	-	2 FN	97 TN

SENSITIVITY = $4/6 = 66\%$

SPECIFICITY = $97/ 170 = 57\%$

PPV = $4/77 = 5.19\%$

NPV = $2/99 = 2.02\%$

DISCUSSION

DISCUSSION

Sex

According to literature, fistula in ano is a male predominant disease. In this study as well, it emerges as a disease primarily affecting males. Only 4.8% out of 228 patients were females.

Age

Although there is no defined age group which has been described as being affected by fistula in ano, in this study it is evident that the disease mostly affects middle aged males. The males in this study were of the range from 12- 69 years with a mean age of 42 years.

Duration of symptoms

Fistula in ano seems to be a chronic disease in our patients, the average duration of symptoms before a patient presented to the institute being 31 months. However, 50 of the total patients had undergone multiple procedures elsewhere. The maximum duration of symptoms recorded was 20 years.

Relation between Number of tracts and number of procedures

Only a small proportion of the patients (25%) of the patients presented with more than one tract. However, the number of tracts did not seem to have a bearing on the number of procedures undergone by the patients as most of the patients who had multiple procedures (three or more) before presentation had single tracts.

Prevalence of tuberculosis

The prevalence of tuberculosis among patients of fistula in ano was found to be 3.07%. This was well within the earlier estimated range of 0.7 % to 21 % of the cases of fistula in ano. (34,35,36,37,38,39). This prevalence does show that tuberculosis is a definite cause of fistula in ano among our populations, and needs to be ruled out with appropriate tests.

However, considering the fact that this institute is a referral center, a higher prevalence might be expected. If this is true, then whether this is a case of under diagnosis is debatable.

The diagnosis of tuberculosis is difficult. The difficulty is even more for the diagnosis of extra pulmonary tuberculosis due to the paucibacillary form of the disease. As a result, the two tests, namely Histopathology and AFB culture, which have been used as the gold standard tests in this study also have their limitations.

Histopathological diagnosis of tuberculosis is usually observed in the presence of granulomatous inflammation and caseous necrosis. In the absence of caseous necrosis, the final diagnosis depends only on the granulomatous inflammation. However, it is not specific for tuberculosis as it can be present in a variety of other conditions such as sarcoidosis, syphilis, leprosy, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, and pneumoconiosis (66).

Histopathology has been found to have a limited sensitivity varying from 46-75% (67,68,69) However, these values represent figures from studies conducted on patients

clinically diagnosed to have tuberculosis. The pretest probability and therefore the validity attributable to these results, therefore, increases. Only 4 out of 231 samples (from 228 patients, none of whom were clinically diagnosed to have tuberculous fistula in ano) were found to be biopsy positive in our study.

Tuberculous PCR has been evaluated in confirming the diagnosis where the biopsy shows granulomas with no demonstrable AFB and in formalin-fixed, paraffin-embedded histologic specimens. These studies have found sensitivity rates from 56-67% with a specificity of about 64% in one such study (70,71,72). However, these studies have used samples which have shown granulomas on biopsies, thus raising the pretest probability of tuberculosis to be likely diagnosis. A positive TB PCR on these specimens can therefore be considered diagnostic of tuberculosis. Another fact that emerged from these studies was that TBPCR was more likely to be positive in cases where the histopathological features were more in favour of tuberculosis. Considering HPE as gold standard, a study evaluating TBPCR has shown 74.1% sensitivity and 96.1% specificity (66). This study could be compared to ours since the samples were not from any specific subset of patients in whom a diagnosis of tuberculosis (clinical or histopathological) was already made. Of the three patients who were found to be biopsy positive in our study, only one was found to be TBPCR positive, which incidentally was also found to be culture positive. The sensitivity of TBPCR for biopsy is therefore only 33% in our study.

The culture for *M. tuberculosis* also has similar problems. It is known to be highly specific (upto 98-100%) but has variable sensitivity from 39% to about 85%. (44,45,46,47). Studies which have compared culture to TBPCR have found sensitivity

85-90% and a specificity of about 98-100% from specimens which were from patients already diagnosed to have tuberculosis. (48,49,50,42,43,44,57,58) In our study, it was found that TBPCR has a sensitivity of 100% and a specificity of only 41% when compared to M. tuberculosis culture.

It has also been shown that the correlation between histopathological and microbiological results can be poor and also be affected by the quality of specimens collected for both. In a retrospective study over 4 years, it was found that tuberculosis was concomitantly diagnosed in only 67% by culture and biopsy whereas, 97% were diagnosed as TB or 'compatible with TB' by histology alone. For 7% of these the final diagnosis was found to be other than TB.(73) In our study, only one of the seven (14%) positive samples was positive for culture and biopsy both. Two samples were diagnosed positive on the basis of biopsy alone and four samples were diagnosed to be positive on the basis of culture alone.

As stated above, 77 patients were PCR positive for the presence of Mycobacterium tuberculosis DNA presented in the samples with only 4 true positives among these. Even though this reflects a high false positive rate, what is striking is that, among the samples which were actually picked up by the PCR, all are culture positive. The rest of the two samples which were not picked up by the PCR were samples which were diagnosed on the basis of histopathology and were finally found to be AFB culture negative.

This makes us consider the fact that the PCR is accurate in amplifying the M. tuberculosis DNA in all the samples when present and showing a positive result. The

question which arises then is, why there were so many (n=73) false positives? This can probably be explained by considering the possible presence of *M. tuberculosis* as a contaminant in the samples, as a part of the resident stool flora in a significant portion of our population. This hypothesis can however be only proven by performing PCR for *M. tuberculosis* on the stool samples of these patients. However, what goes against this hypothesis is a study which used stool from normal patients or patients treated for tuberculosis as controls for TBPCR and found all specimens (n=30) to be negative for the same (i.e. a specificity of 100%).(59) Our study shows a specificity of 57%.

Another factor which can be considered to explain the high false positive rate could be due to carry-over contamination during collection, storage, or processing. (66) In the present study, different steps of PCR (DNA extraction, pre-PCR mixing, PCR, and post-PCR gel documentation) were conducted in different rooms, reducing the chances of contamination.

It must also be kept in mind that even though culture and biopsy are not tests with a wide and rather poor range of sensitivity, they have been considered the gold standard tests for the lack of any other test. It shall therefore not be unwise to keep in mind the possibility that had the gold standard tests been more sensitive, the number of cases diagnosed to have definite tuberculous fistula in ano would have been higher and therefore the number of false positives would have been lower.

Another possible explanation to account for the false positive results would be that in these cases the disease was still developing and well-developed granuloma or numbers had not yet formed, or bacillary load was not enough for culture to show

positive results but enough to be detected by PCR. Whether this is true can be ascertained by two ways ; 1) By following up these patients in time to determine how many of them develop recurrence or resistance to conventional methods of treatment or 2) By empirically treating them with ATT and observe them for healing of these fistulae.(66) To start treatment on the basis of positive TBPCR may not be completely acceptable given the low pretest probability of these patients to have tuberculosis.

Out of the 228 patients who were included in this study, one patient was found to have an adenocarcinoma. This is consistent with some studies which show that chronic fistula in an can be associated with mucinous adenocarcinoma.(74,75)

Only one case among 228 patients was diagnosed to have Crohn's disease on a histological basis.

Another fact which was evident in the above study was the fact that none of the patients who were diagnosed to have tuberculosis were diagnosed to have concomitant pulmonary tuberculosis. This is in contradiction to the earlier belief some studies which have suggesting a definitive association between the two.(76)

CONCLUSIONS

CONCLUSIONS

1. Tuberculosis is an rare but definite cause of fistula in ano. It should be actively ruled out by testing specimens by histopathological as well as microbiological (Culture) means.
2. Fistula in ano is a chronic disease affecting mostly males.
3. Tuberculous fistula in ano can exist as an entity independent from pulmonary or other systemic involvement of the disease.
4. TBPCR does not appear to be an useful test in diagnosing tuberculous fistula in ano due to a high rate of false positive results. However, in culture positive samples, TBPCR is 100% sensitive in detecting the same.

LIMITATIONS OF THE STUDY

1. Delay in processing the samples after collection may have altered the results.
2. The possibility of carry over contamination could not be ruled out, thus casting a doubt on the interpretation of the results in light of the high false positive rates.

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